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Review article

Divergent modulation of normal and neoplastic stem cells by thrombospondin-1 and CD47 signaling

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ABSTRACT

Thrombospondin-1 is a secreted matricellular protein that regulates the differentiation and function of many cell types. Thrombospondin-1 is not required for embryonic development, but studies using lineage-committed adult stem cells have identified positive and negative effects of thrombospondin-1 on stem cell differentiation and self-renewal and identified several thrombospondin-1 receptors that mediate these responses. Genetic studies in mice reveal a broad inhibitory role of thrombospondin-1 mediated by its receptor CD47. Cells and tissues lacking thrombospondin-1 or CD47 exhibit an increased capacity for self-renewal associated with increased expression of the stem cell transcription factors c-Myc, Sox2, Klf4, and Oct4. Thrombospondin-1 inhibits expression of these transcription factors in a CD47-dependent manner. However, this regulation differs in some neoplastic cells. Tumor initiating/cancer stem cells express high levels of CD47, but in contrast to nontransformed stem cells CD47 signaling supports cancer stem cells. Suppression of CD47 expression in cancer stem cells or ligation of CD47 by function blocking antibodies or thrombospondin-1 results in loss of self-renewal. Therefore, the therapeutic CD47 antagonists that are in clinical development for stimulating innate anti-tumor immunity may also inhibit tumor growth by suppressing cancer stem cells. These and other therapeutic modulators of thrombospondin-1 and CD47 signaling may also have applications in regenerative medicine to enhance the function of normal stem cells.

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Abbreviations: CFU, colony-forming units; CSCs, cancer stem cells; EBs, embryoid bodies; ECM, extracellular matrix; EPCs, endothelial progenitor cells; ESC, embryonic stem cells; HSC, hematopoietic stem cells; iPS, inducible pluripotent stem; MSCs, mesenchymal stem cells; NO, nitric oxide; NOD, nonobese diabetic; SIRPα, signal regulatory protein-α; SSEA1, stage-specific embryonic antigen-1; TGF-β, transforming growth factor-β1; TSP1, thrombospondin-1; TSP2, thrombospondin-2.

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1. Introduction

The concept that stem cells can give rise to both normal and malignant tissues has attracted growing interest in developing therapeutics to target these cells. Pluripotent cells were first identified in mouse embryos in 1981 (Evans and Kaufman, 1981), and a mouse teratocarcinoma stem cell line was established the same year (Martin, 1981). Pluripotent stem cell lines were first generated from pigs and sheep (Notarianni et al., 1991). Blastocysts produced by in vitro fertilization for clinical purposes were used to develop the first human embryonic stem cell (ESC) line (Thomson et al., 1998). One approach to circumvent the ethical issues surrounding the use of human embryos for regenerative medicine was to create induced pluripotent stem (iPS) cells using somatic cells isolated from adult tissues. Routine creation of iPS cells was enabled by identification of the four critical stem cell transcription factors cMyc, Sox2, Oct3/4 and Klf4 (Takahashi and Yamanaka, 2006). Forced expression of these four proteins is sufficient to convert various somatic cells into iPS cells. However, the potential of transplanted iPS cells to form teratomas or teratocarcinomas in patients spurred efforts to develop lineage-committed adult stem cells that lack this potential.

Stem cells are also of growing interest in cancer research. Cancers may arise by transformation of tissue stem cells, or transformed somatic cells may activate the self-renewal program of stem cells (Pardal et al., 2003). Regardless of their origin, it is clear that many tumors are sustained by a minor population of tumor initiating cells that share many properties of stem cells. In this review, we examine the role of the matricellular protein thrombospondin-1 (TSP1) and its receptors in stem cell biology, focusing on how TSP1 interactions with its receptors, including CD47, differentially modulate stem cell physiology in normal and neoplastic cells.

2. Stem cells and extracellular matrix

The concept of a stem cell niche encompasses supporting cells, which provide specific cues needed to regulate quiescence of stem cells and maintain their asymmetric division, and a specialized niche extracellular matrix (ECM) that releases growth factors and engages specific ECM receptors on stem cells to control their fate. Signals provided by these ECM receptors mediate dynamic communication between ESCs and their niche (Gattazzo et al., 2014). This ECM is composed of soluble and bound macromolecules that facilitate three-dimensional assembly of stem cells and supporting cells that provides organizing and signaling cues. Changes in stem cell-ECM interactions provide environmental cues that guide wound healing, homeostasis, aging, and stem cell maintenance (Discher et al., 2009; Watt and Fujiwara, 2011).

Thrombospondins are a family of five secreted proteins in vertebrates (Adams and Lawler, 2011). Some thrombospondins are constitutive elements of ECM, but TSP1 and thrombospondin-2 (TSP2) are matricellular proteins that are not constitutive in ECM but are expressed transiently under specific conditions, where they alter cell behavior and ECM remodeling via their interactions with growth factors in the ECM and by engaging specific transmembrane receptors including proteoglycans, integrins, and the nonintegrin receptors CD36, CD47, and CD148 (Calabro et al., 2014; Roberts et al., 2012; Takahashi et al., 2012).

CD47 is a signaling receptor for TSP1 that is ubiquitously expressed, but at a higher level on some stem cells. CD47 consists of an extracellular IgV domain followed by five membrane-spanning segments and a short variably spliced C-terminal cytoplasmic tail (Soto Pantoja et al., 2013). TSP1 binding to CD47 results in cell type-specific signaling that can alter cell adhesion, motility, growth, differentiation, and survival (Oldenborg, 2013; Soto-Pantoja et al., 2015) (Fig. 1). Some of these signals are mediated by lateral interactions of CD47 with specific integrins and other signaling receptors in the plasma membrane. CD47 also serves as the counter-receptor for signal regulatory protein-α (SIRPα) and SIRPγ (Barclay and Van den Berg, 2014; Matozaki et al., 2009). SIRPα is highly expressed on phagocytes, and binding of the IgV domain of CD47 on a target cell to the IgV domain of SIRPα on macrophages elicits signaling mediated by the phosphatase SHP1 that blocks phagocytosis of the target cell (Fig. 1) (Oldenborg et al., 2000). Accumulating evidence indicates that CD47 expressed by stem cells serves both as a cell-autonomous signaling receptor and as a SIRPα counter-receptor to limit stem cell clearance. Although less explored in stem cells, SIRPα binding could potentially elicit signaling through CD47 that may be distinct from that produced by TSP1 binding.

3. TSP1 in hematopoietic stem cell differentiation and self-renewal

A role for TSP1 in hematopoietic stem cells (HSCs) was first reported in 1990 (Long and Dixit, 1990). Nonadherent low density human bone marrow cells were shown to adhere specifically on immobilized TSP1. This was true for mixed-lineage progenitors as well as those from colonies containing erythroid burst-forming cells (BFU-E), and erythroid, granulocyte/macrophage, and megakaryocyte colony-forming cells (CFU-GEMM). This activity was mapped to a large C-terminal region of TSP1 and shown to be inhibited by specific monoclonal antibodies that bind to this domain of TSP1. A subsequent report in 1992 reproduced these results using sorted $CD34^+DR^-CD15^-$ human bone marrow cells that exhibited characteristics of HSCs including self-renewal and the ability to differentiate into multiple hematopoietic lineages (Long et al., 1992). The $CD34^+DR^-CD15^-$ hematopoietic progenitor cells attached on TSP1 but not on immobilized fibronectin. In combination with c-Kit, TSP1 was shown to function as a colony-stimulating factor for $CD34^+DR^-CD15^-$ progenitors. In contrast, TSP1 inhibited colony formation driven by the cytokine IL-3. This suggested that TSP1 is a context-dependent positive and negative regulator of HSC differentiation. Another early study using murine progenitor cells showed that TSP1 significantly inhibited murine megakaryocytopoiesis at a concentration of 1 μg/ml (2.2 nM) (Chen et al., 1997). In contrast to the stimulatory activity described above, this inhibitory activity was reproduced by a recombinant N-terminal domain of TSP1. TSP1 was further shown to inhibit the growth of multipotent hematopoietic colony-forming units (CFU-GEMM) but not those committed to granulocyte/macrophage (CFU-GM) or erythroid (BFU-E) lineages. Studies using a CD36 antibody that inhibits TSP1 binding suggested that the TSP1-induced inhibition of megakaryocytopoiesis is mediated in part by the binding of TSP1 to its receptor CD36 expressed on the megakaryocytic progenitors (Yang et al., 2003). Notably, the N-terminal domain of TSP1 does not contain its CD36-binding site, so it is unclear how the results of these two studies can be rationalized.

4. Thrombospondins in other adult stem cells

Recombinant human TSP1 had a negative effect on the angiogenic potential of human endothelial colony-forming stem cells (Smadja et al., 2011). Suppression of either TSP1 expression or CD47 expression in the endothelial colony-forming cells using siRNA enhanced their angiogenic potential, implicating CD47 as the inhibitory TSP1 receptor in these stem cells. Consistent with the known role of TSP1/CD47 signaling in limiting vascular cell nitric oxide (NO)/cGMP signaling (Soto-Pantoja et al., 2015),